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[Orn^5]URP acts as a pure antagonist of urotensinergic receptors in rat cortical astrocytes

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ABSTRACT

Cultured rat astrocytes, which express functional urotensin II (UII)/UII-related peptide (URP) receptors (UT), represent a very suitable model to investigate the pharmacological profile of UII and URP analogs towards native UT. We have recently designed three URP analogs [D-Trp^4]URP, [Orn^5]URP and [D-Tyr^6]URP, that act as UT antagonists in the rat aortic ring bioassay. However, it has been previously reported that UII/URP analogs capable of inhibiting the contractile activity of UII possess agonistic activity on UT-transfected cells. In the present study, we have compared the ability of URP analogs to compete for [^{125}I]URP binding and to modulate cytosolic calcium concentration ([Ca^{2+}]$_c$) in cultured rat astrocytes. All three analogs displaced radioligand binding: [D-Trp^4]URP and [D-Tyr^6]URP interacted with high- and low-affinity sites whereas [Orn^5]URP only bound high-affinity sites. [D-Trp^4]URP and [D-Tyr^6]URP both induced a robust increase in [Ca^{2+}]$_c$ in astrocytes while [Orn^5]URP was totally devoid of activity. [Orn^5]URP provoked a concentration-dependent inhibition of URP- and UII-evoked [Ca^{2+}]$_c$ increase and a rightward shift of the URP and UII dose–response curves. The present data indicate that [D-Trp^4]URP and [D-Tyr^6]URP, which act as UII antagonists in the rat aortic ring assay, behave as agonists in the [Ca^{2+}]$_c$ mobilization assay in cultured astrocytes, whereas [Orn^5]URP is a pure selective antagonist in both rat aortic ring contraction and astrocyte [Ca^{2+}]$_c$ mobilization assays.

1. Introduction

Urotensin II (UII) is a cyclic peptide initially isolated from the caudal neurosecretory system of teleost fish [30]. UII has been subsequently identified in the brain of amphibians [11] and mammals [13,14]. Recently, a paralog of UII termed UII-related peptide (URP, Table 1) has been characterized in mouse, rat and human [35]. Comparative genomics studies have shown that UII and URP are two members of a family of regulatory peptides that also comprises somatostatin and cortistatin [36]. Indeed, all four peptides possess a disulfide bridge and share, within their cyclic region, the Phe-Trp-Lys motif that is essential for their biological activities [12,36].

UII and URP are the endogenous ligands of the previously orphan G-protein-coupled receptor GPR 14 [2,23,27,29,35] now renamed UT receptor. Exposure of UT-transfected cells to UII

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Abbreviations: [Ca^{2+}]$_c$, cytosolic calcium concentration; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GPCR, G-protein-coupled receptor; PBS, phosphate-buffered saline; UII, urotensin II; URP, urotensin II-related peptide; UT, UII receptor.

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or URP causes activation of phospholipase C and an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_c$) [16,35]. The UT receptor is most similar to members of the somatostatin receptor family, notably sst4 [25], and it has been shown that somatostatin and cortistatin can, to a certain extent, increase $[Ca^{2+}]_c$ in UT-transfected cells [23]. Based on the structural homology between somatostatin receptors and UT, it is likely that the Lys residue within the cyclic region of UII and URP interacts with the conserved Asp residue in transmembrane domain 3 of UT, as previously shown for somatostatin and its receptors [28,34].

Structure–activity relationship studies have shown the importance of the conserved hexapeptide ring of UII and URP in the biological activity of both peptides [22]. In particular, point substitution of each individual amino acid of the Trp-Lys-Tyr tripeptide by their D-enanthiomers suppresses the binding affinity and/or the contractile activity of UII [21] and URP [9]. Concurrently, molecular modeling under NMR constraints has revealed the occurrence of an inverse γ-turn centered on the Trp^4-Lys^5-Tyr^6 triad of URP that is likely to play a crucial role in the biological activity of the peptide [9].

So far, the signaling mechanisms associated with UT activation have mainly been investigated on cell lines transfected with recombinant receptors [1,16,33,37]. Recently, we have found that cultured rat astrocytes constitutively express the UT gene and possess high-affinity UII binding sites [8]. Exposure of cultured astrocytes to UII activates phospholipase C and increases $[Ca^{2+}]_c$ in a concentration-dependent manner [8]. Cultured rat astrocytes thus represent a suitable model in which to investigate the pharmacological characteristics of UII and URP analogs towards native UT receptors.

Owing to the potential implication of the urotensinergic system in pathophysiological conditions, notably in renal and cardiovascular diseases, several groups are currently trying to develop selective UT antagonists [3,5,10]. In this context, we have recently designed three URP analogs (Table 1) that were found to decrease ([D-Tyr^6]URP) or suppress ([D-Trp^4]URP and [Orn^5]URP) UII-evoked contraction of rat aortic rings [9]. Paradoxically, some antagonists devoid of intrinsic contractile activity may behave as agonists at recombinant receptors, as determined by measuring their ability to induce calcium mobilization [4,6,7]. It is thus necessary to characterize the pharmacological profile of potential antagonists not only on UT-transfected cells but also on cells expressing native UT receptors. The aim of the present study was to examine the ability of [D-Trp^4]URP, [Orn^5]URP and [D-Tyr^6]URP to interact with naturally occurring UII binding sites in rat cortical astrocytes [8] and to investigate their agonistic and antagonistic activities by using a calcium mobilization assay.

2. Materials and methods

2.1. Reagents

URP (ACFWKYCV), rat UII (UII, pQHGTAPECFWKYCI) and the URP analogs [D-Trp^4]URP, [Orn^5]URP and [D-Tyr^6]URP were synthesized by the solid phase methodology on a Pioneer PerSeptive Biosystem peptide synthesizer (Applera, Courtaboeuf, France) using the standard Fmoc procedure as previously described [9,21]. All peptides were purified (>98%) on a 2.2 cm × 25 cm Vydac C_{18} column (Alltech, Templemars, France) and characterized by MALDI-TOF MS on a Voyager DE-PRO mass spectrometer (Applera). Glutamine, *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid (HEPES), probenecid and the antibiotic–antimycotic solution were purchased from Invitrogen (Cergy-Pontoise, France). Dulbecco's modified Eagle's medium (DMEM), Ham-F12 culture medium, insulin, D(+) glucose and bovine serum albumin (BSA) were obtained from Sigma (St-Quentin Fallavier, France). Fetal bovine serum (FBS) was from Eurobio (Les Ulis, France). Pluronic acid and fluo-4-acetoxymethyl ester (fluo-4-AM) were from Molecular Probes (Leiden, The Netherlands).

2.2. Cell culture

Primary cultures of astrocytes were prepared as previously described [19]. Briefly, cerebral hemispheres from newborn Wistar rats were collected in DMEM/Ham-F12 (2:1, v/v) culture medium supplemented with 2 mM glutamine, 1% insulin, 5 mM HEPES, 0.4% glucose and 1% of the antibiotic–antimycotic solution. The tissues were disaggregated mechanically with a syringe equipped with a 1-mm gauge needle, and filtered through a 100-μm pore size mesh filter (Falcon, Becton Dickinson, Grenoble, France). Dissociated cells were resuspended in culture medium supplemented with 10% heat-inactivated FBS and seeded in 150-cm^2 culture flasks (Falcon) at a density of 2×10^7 cells per flask. The cells were incubated at 37 °C in a humidified atmosphere (5% CO_2) and the medium was changed twice a week. When cultures were confluent, the flasks were gently shaken on an orbital shaker at 250 rpm for 2 h. Dislodged cells were discarded and a second step of purification was performed at 250 rpm for 14–16 h. Remaining adhesive cells were collected by trypsination, centrifuged ($100 \times g$, 10 min) and plated in 150-cm^2 flasks. Suspended astrocytes were harvested and seeded in complete medium supplemented with 10% FBS in poly-L-lysine-coated 24-well plates at 80,000 cells/well (for binding studies on dried cultured cells) or in 96-well plates at 30,000 cells/well (for calcium mobilization assay). The purity of the cultures was assessed by cytometry after GFAP immunostaining. The enriched cultures contained >98% astrocytes [8].

2.3. Competition studies

Three micrograms of URP in phosphate buffer (0.375 mM, pH 7.4) was labeled with 0.5 mCi $Na^{125}I$ (Amersham Biosciences, Orsay, France) by the lactoperoxidase method as previously described [9]. Mono-iodinated [^{125}I]URP used as a radioligand for binding assay was purified by reversed-phase HPLC on an adsorbosphere C_{18} column (0.46 cm × 25 cm, Alltech) using a linear gradient (25–65% over 40 min) of acetonitrile/trifluoro acetic acid (TFA, 99.9:0.1, v/v) at a flow rate of 1 ml/min, and stored at 4 °C. The specific radioactivity of the tracer was approximately 2000 Ci/mmol. After 2 days in culture, astrocytes were rinsed three times with phosphate-buffered saline (PBS), dried under a cold air stream and stored at

−80 °C until binding experiments. Frozen cells were washed twice with assay buffer (50 mM Tris buffer, 1 mM $MnCl_2$ and 0.5% BSA) and incubated at 22 °C for 3 h in the same buffer with [^{125}I]URP (0.1 nM) in the presence of graded concentrations of unlabeled [D-Trp^4]URP, [Orn^5]URP or [D-Tyr^6]URP. At the end of the incubation, cells were washed three times with assay buffer, solubilized with 1% SDS, and the radioactivity was counted in a gamma counter (LKB Wallac, Evry, France). Non-specific binding was determined by addition of 1 μM unlabeled URP.

2.4. *Calcium mobilization assay*

After 2 days in culture, astrocytes were rinsed twice with 90 μl of a modified Hank's balanced salt solution (HBSS) containing (in mM): 135 NaCl; 5 KCl; 1 $MgCl_2$; 1 $CaCl_2$; 10 HEPES, 3 glucose; 2.5 probenicid (pH 7.3). Cells were incubated at 37 °C with 40 μl of 2 μM fluo-4-AM dye containing 20% pluronic acid for 40 min in a 5% CO_2 atmosphere. Cells were washed twice with 90 μl modified HBSS, and 200 μl HBSS was added. Cells were incubated for 30 min in the absence or presence of [Orn^5]URP and the effect of graded concentrations of URP and UII on $[Ca^{2+}]_c$ was measured with a fluorometric imaging plate reader FlexStation (Molecular Devices, Sunnyvale, CA).

2.5. *Data analysis*

For competition studies, the Hill coefficient (n_H) was calculated from the competition curves fitted to the Hill equation *via* a nonlinear regression using the computerized curve-fitting package GraphPad Prism version 4 for Windows (GraphPad Software, San Diego, CA). The data were then re-fitted using equation for displacement of radioligand by competitors to one or two binding sites. A two-site curve-fitting model was selected when this model fitted the data significantly better than a one-site model, as determined by a *F*-test at a significance level of $P < 0.05$. IC_{50} values derived from these latter fits were converted to apparent competition-receptor dissociation equilibrium constants K_1 for the high-affinity and K_2 for the low-affinity binding sites (Table 1, Fig. 1).

For calcium mobilization assays, the Student's *t*-test and one-way ANOVA followed by a Dunnett's post hoc test were used to compare the intrinsic activity of the URP analogs (Fig. 2). For the antagonistic activity of [Orn^5]URP, the dose–response curves were fitted to a sigmoidal concentration–response using the fitting routine GraphPad Prism. pK_b was determined from a plot representing the decrease in calcium mobilization evoked by [Orn^5]URP on the $[Ca^{2+}]_c$ increase induced by 10 nM URP and UII (Fig. 3). For the Schild analysis,

Table 1 – Amino acid sequence of rUII, URP and the three URP analogs studied in the present report

Peptide name	Sequence
rUII	*p*Glu-His-Gly-Thr-Ala-Pro-Glu-Cys-Phe-Trp-Lys-Tyr-Cys-Ile-OH
URP	H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH
[D-Trp^4]URP	H-Ala-Cys-Phe-***Trp***-Lys-Tyr-Cys-Val-OH
[Orn^5]URP	H-Ala-Cys-Phe-Trp-Orn-Tyr-Cys-Val-OH
[D-Tyr^6]URP	H-Ala-Cys-Phe-Trp-Lys-***Tyr***-Cys-Val-OH

D-Amino acids are indicated in bold italic letters.

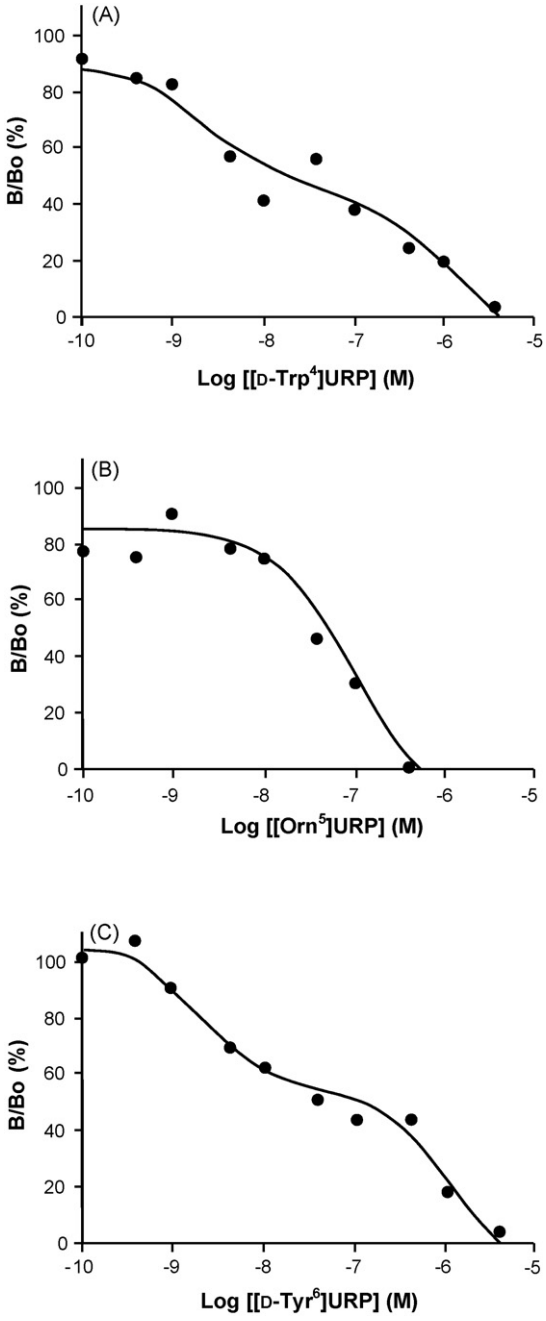

Fig. 1 – Competition curves comparing displacement of [^{125}I]URP binding to cultured rat cortical astrocytes by the three URP analogs [D-Trp^4]URP, [Orn^5]URP and [D-Tyr^6]URP. Cells were incubated for 3 h at 22 °C with 0.1 nM [^{125}I]URP in the absence or presence of graded concentrations of [D-Trp^4]URP (A, 100 pM to 3 μM), [Orn^5]URP (B, 100 pM to 0.3 μM) and [D-Tyr^6]URP (C, 100 pM to 3 μM). Each value represents the mean of four independent experiments performed in triplicate.

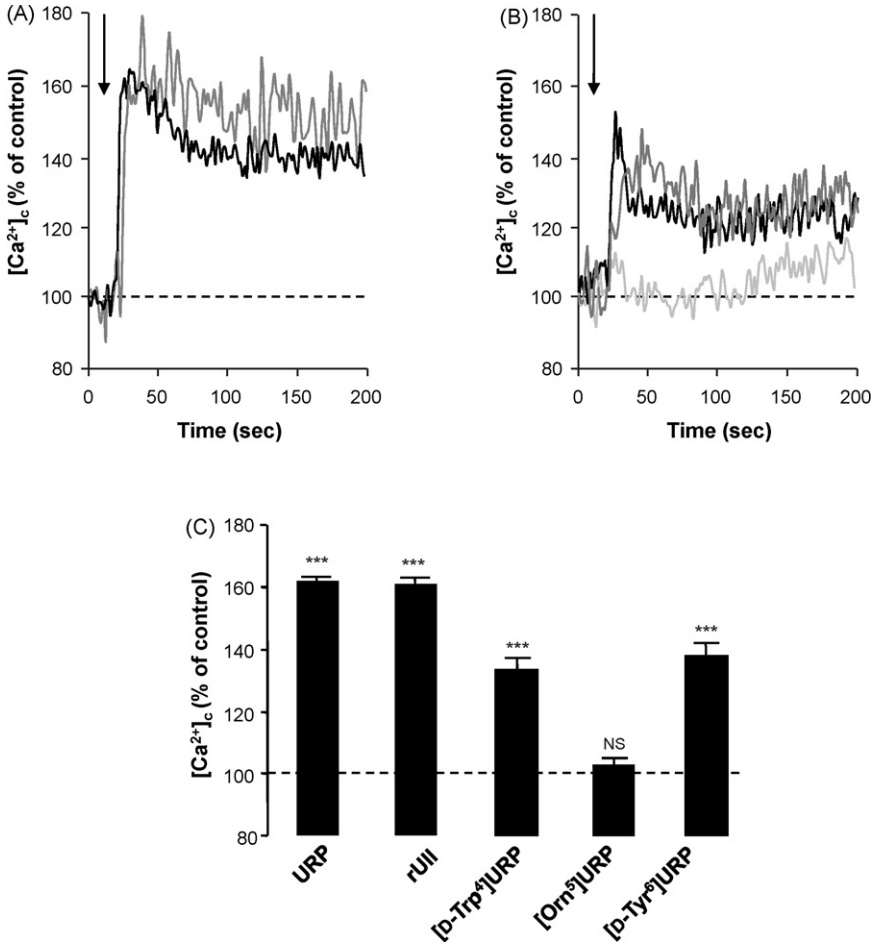

Fig. 2 – Effect of URP, rUII and URP analogs on $[Ca^{2+}]_c$ in cultured rat cortical astrocytes. (A) Representative recordings of cells exposed (arrow) to URP (▬) or rUII (▬) (10 nM each). (B) Representative recordings of cells exposed (arrow) to [D-Trp^4]URP (▬), [Orn^5]URP (▬) or [D-Tyr^6]URP (▬) (10 μM each). (C) Quantification of the effects of URP, rUII and the URP analogs on $[Ca^{2+}]_c$ in astrocytes. Each value represents the maximum amplitude of $[Ca^{2+}]_c$ transients induced by URP, rUII or the URP analogs. The results are expressed as percentages of the corresponding control values in the absence of the peptides. Data are mean ± S.E.M. of at least eight independent experiments. ***P < 0.001; NS: not statistically different from the control (ANOVA followed by Dunnett post hoc test).

the concentration ratios (CR) were calculated from individual dose–response curves of URP and UII obtained with graded concentrations of [Orn^5]URP (Fig. 4).

3. Results

3.1. *Effect of the URP analogs on [^{125}I]URP binding*

The [D-Trp^4]URP, [Orn^5]URP and [D-Tyr^6]URP analogs were tested for their ability to compete with [^{125}I]URP binding on purified cultured cortical astrocytes (Fig. 1). The results showed that the three compounds displaced the radioligand from its binding sites. Statistical analysis using the *F*-test revealed that [D-Trp^4]URP and [D-Tyr^6]URP, as URP itself, interact with high- (K_1) and low-affinity (K_2) sites ([D-Trp^4]URP: $K_1 = 6.81 \pm 2.67$ nM, $K_2 = 6.05 \pm 2{,}02$ μM; [D-Tyr^6]URP: $K_1 = 2.35 \pm 1.50$ nM, $K_2 = 0.56 \pm 0.28$ μM; URP: $K_1 = 0.19 \pm 0.04$ nM, $K_2 = 0.65 \pm 0.19$ μM). In contrast, [Orn^5]URP was only able to bind high-affinity sites but with a weaker potency than the other analogs ($K = 67.7 \pm 38.9$ nM) (Fig. 1 and Table 2). The values of Hill coefficient (n_H) confirmed that URP ($n_H = 0.49 \pm 0.08$) or the analogs [D-Trp^4]URP ($n_H = 0.72 \pm 0.10$) and

Table 2 – Competition binding parameters for URP and URP analogs on cultured rat cortical astrocytes

Analog	K_1(nM)	K_2(μM)	n_H
URP	0.19 ± 0.04	0.65 ± 0.19	0.49 ± 0.08
[D-Trp^4]URP	6.81 ± 2.67	6.05 ± 2.02	0.72 ± 0.10
[Orn^5]URP	67.7 ± 38.9	–	0.98 ± 0.24
[D-Tyr^6]URP	2.35 ± 1.50	0.56 ± 0.28	0.59 ± 0.11

Cells were incubated for 3 h with 0.1 nM [^{125}I]URP in the presence of graded concentrations of URP, [D-Trp^4]URP, [Orn^5]URP or [D-Tyr^6]URP (100 pM to 3 μM, each). Each value represents the mean ± S.E.M. of four independent experiments performed in triplicate. K_1 and K_2: dissociation constants; n_H: Hill coefficient.

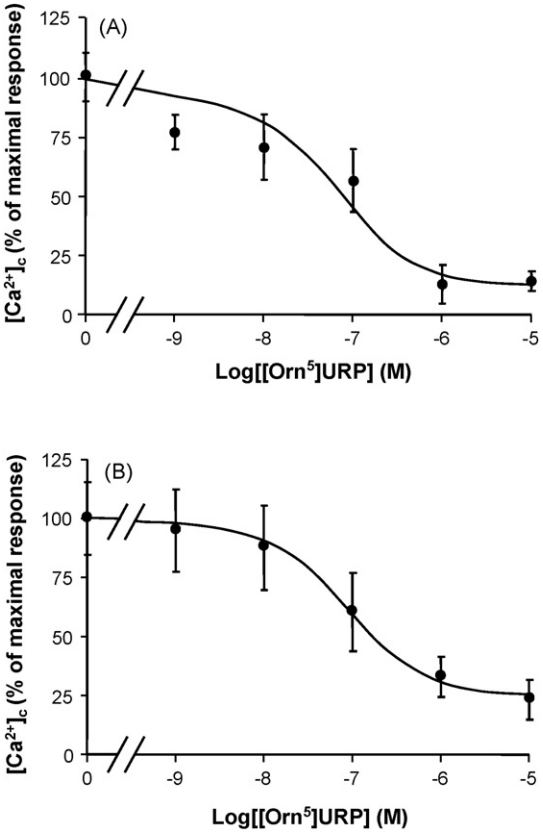

Fig. 3 – Effect of graded concentrations of [Orn^5]URP on the $[Ca^{2+}]_c$ increase induced by URP (A, 10 nM) or rUII (B, 10 nM) in cultured rat cortical astrocytes. Each point represents the maximum amplitude of $[Ca^{2+}]_c$ transients induced by URP (A) or rUII (B). The results are expressed as percentages of the calcium mobilization provoked by 10 nM URP (A) or rUII (B). Data are mean ± S.E.M. of at least eight independent experiments.

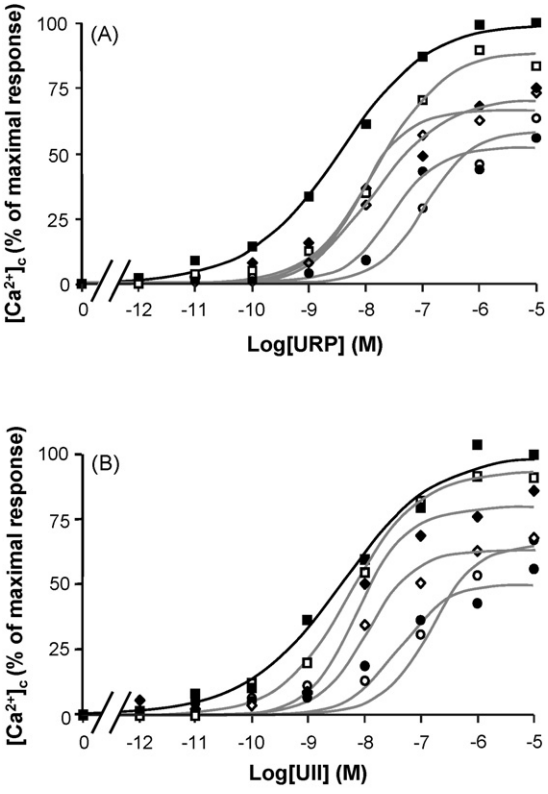

Fig. 4 – Effect of [Orn^5]URP on URP- and rUII-induced $[Ca^{2+}]_c$ increase in cultured rat cortical astrocytes. Cells were exposed to graded concentrations of URP (A) or rUII (B) in the absence (■) or presence of [Orn^5]URP ((□) 1 nM; (◆) 10 nM; (◇) 100 nM; (○) 1 μM; (●) 10 μM). Each point represents the maximum amplitude of $[Ca^{2+}]_c$ transients induced by URP or rUII. The results are expressed as percentages of the calcium mobilization provoked by 10 μM URP (A) or rUII (B). Data are mean of at least eight independent experiments.

[D-Tyr^6]URP ($n_H = 0.59 \pm 0.11$) bind a heterogeneous population of binding sites whereas [Orn^5]URP ($n_H = 0.98 \pm 0.24$) interacts with a single population of recognition sites.

3.2. *Effect of the URP analogs on $[Ca^{2+}]_c$*

Exposure of astrocytes to [D-Trp^4]URP or [D-Tyr^6]URP (10 μM, each) provoked a robust and sustained increase in $[Ca^{2+}]_c$ (+33 ± 4% and +37 ± 4%, respectively) in very much the same as URP (10 nM; +61 ± 5%) and UII (10 nM; +60 ± 9%) (Fig. 2). In contrast, [Orn^5]URP (10 μM) did not affect $[Ca^{2+}]_c$ in astroglial cells (Fig. 2). Pretreatment of astrocytes with graded concentrations of [Orn^5]URP (1 nM to 10 μM) dose-dependently inhibited the $[Ca^{2+}]_c$ rise induced by both URP and UII ($pIC_{50} = 7.12 \pm 0.32$ and 7.34 ± 0.43, respectively) (Fig. 3). Graded concentrations of [Orn^5]URP caused a rightward shift of the URP and UII dose–response curves associated with a decrease in the maximal responses (Fig. 4). Schild analysis yielded a regression line slope significantly lower than 1 for both URP (0.47 ± 0.03; $P < 0.01$) and UII (0.56 ± 0.05; $P < 0.01$), indicating that [Orn^5]URP behaves as a non-competitive/insurmountable antagonist (data not shown).

4. Discussion

The cyclic core sequence of UII and URP, which has been strongly preserved during evolution [9,36], plays a crucial role in the binding affinity at the UT receptor and the biological activity of the two peptides [9,18,20,21]. We have recently designed three URP analogs, [D-Trp^4]URP, [Orn^5]URP and [D-Tyr^6]URP, which act as UT antagonists in the rat aortic ring assay [9]. Since it has been previously reported that UII/URP analogs that inhibit the contractile activity of UII on rat aorta can behave as agonists on UT-transfected cells, in the present study we have investigated the pharmacological profile of the three aforementioned analogs by using cultured rat astrocytes, a cell model that express functional UT receptors [8].

We found that all three analogs were able to displace [^{125}I]URP binding on cultured astrocytes, but generated distinct competition curves. Thus, the profile of the dissociation curve obtained with [Orn^5]URP was strictly monophasic with a Hill coefficient close to unity and a dissociation constant similar to that reported on UT-transfected CHO cells [9]. In contrast, the dissociation curves obtained with [D-Trp^4]URP and [D-Tyr^6]URP

were biphasic and the Hill coefficients were clearly below 1, indicating that these two analogs can recognize a heterogeneous population of binding sites. In agreement with these data, the existence of two binding sites for UII has recently been demonstrated in rat cortical astrocytes [8] and in CHO cells transfected with human UT [10]. While the high-affinity site most likely corresponds to UT, the molecular structure of the low-affinity site is currently a matter of speculation. UII and URP belong to the same gene family as somatostatin and cortistatin [36] and all four peptides share substantial sequence identity in their cyclic core sequence [12]. In addition, UT and somatostatin receptors are closely related [25]. Since rat cortical astrocytes express three somatostatin receptor subtypes, *i.e.* sst1, sst2 and sst4 [17,26], it might be hypothesized that [Orn^5]URP binds two receptor sites, *i.e.* UT and somatostatin receptor(s), with the same affinity. These somatostatin receptor(s) may correspond to the low-affinity site recognized by [D-Trp^4]URP and [D-Tyr^6]URP. In support of this hypothesis, it has been recently demonstrated that UII and URP can both activate the porcine somatostatin receptors sst2 and sst5 [24]. Alternatively, the two affinity sites could be accounted for by the occurrence of both monomeric, dimeric and/or oligomeric forms of UT with different binding affinities. Consistent with this latter hypothesis, Clozel et al. [10] have previously described the existence of two distinct affinity sites in UT-transfected CHO cells which do not express any somatostatin receptors.

The present study has shown that [D-Trp^4]URP and [D-Tyr^6]URP, but not [Orn^5]URP, provoke a robust increase in $[Ca^{2+}]_c$ in cultured astrocytes, indicating that [D-Trp^4]URP and [D-Tyr^6]URP behave as agonists in our model. Several lines of evidence suggest that the increase in $[Ca^{2+}]_c$ induced by these two URP analogs is mediated through UT: (i) stimulation of UT in UT-transfected cells [2,9,10,35] or in cells expressing native UT [8,31,32] is known to activate the PLC/PKC pathway leading to an increase in $[Ca^{2+}]_c$; (ii) we have recently shown that rat astrocytes express the UT mRNA and the UT protein [8]; and (iii) the stimulatory effect of [D-Trp^4]URP and [D-Tyr^6]URP on $[Ca^{2+}]_c$ in rat astrocytes is mimicked by URP and UII, two natural ligands of UT. However, the $[Ca^{2+}]_c$ increase evoked by [D-Trp^4]URP and [D-Tyr^6]URP could be also mediated in part through activation of somatostatin receptors since a recent study has shown that URP and UII provoke an elevation of $[Ca^{2+}]_c$ in CHO cells stably expressing the porcine somatostatin receptors sst2 and sst5 [24]. In support of this hypothesis, it has been previously reported that somatostatin increases $[Ca^{2+}]_c$ in mouse striatal astrocytes [15].

The [Orn^5]URP analog, which was devoid of agonistic activity on Ca^{2+} mobilization in cultured rat astrocytes, inhibited in a dose-dependent manner the $[Ca^{2+}]_c$ increase induced by URP and UII. Graded concentrations of [Orn^5]URP shifted to the right of the dose–response curves of URP and UII, and induced a concomitant decrease of the maximum responses. Shild analysis revealed that, for both URP and UII, the slopes of the regression lines were significantly lower than one, indicating that [Orn^5]URP behaves as an insurmountable/non-competitive antagonist in rat astrocytes. Another UII analog modified at the Lys residue, [Pen^5,D-Trp^7,Dab^8]UII$_{4\text{-}11}$ (UFP-803), has previously been shown to act as a non-competitive antagonist in the Ca^{2+} mobilization assay and a competitive antagonist in the aorta ring assay [7]. These observations confirm the crucial role of the Lys residue in the cyclic domain of URP and UII [22]. Whether [Orn^5]URP acts as a competitive or non-competitive antagonist in the isolated rat aorta remains to be investigated.

In conclusion, the present study has shown that the two URP analogs [D-Trp^4]URP and [D-Tyr^6]URP, which inhibit UII-induced contraction of isolated rat aorta, behave as agonists in the calcium mobilization assay in cultured rat astrocytes. In contrast, the URP analog [Orn^5]URP, which also inhibits the action of UII in the rat aorta ring assay, behaves as a potent insurmountable/non-competitive UT antagonist in rat astrocytes. [Orn^5]URP may thus be a useful tool to investigate the functional role of UT in astroglial cells.

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REFERENCES

[1] Aiyar N, Johns DG, Ao Z, Disa J, Behm DJ, Foley JJ, et al. Cloning and pharmacological characterization of the cat urotensin-II receptor (UT). Biochem Pharmacol 2005;69:1069–79.
[2] Ames RS, Sarau HM, Chambers JK, Willette RN, Aiyar NV, Romanic AM, et al. Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. Nature 1999;401:282–6.
[3] Behm DJ, Herold CL, Camarda V, Aiyar NV, Douglas SA. Differential agonistic and antagonistic effects of the urotensin-II ligand SB-710411 at rodent and primate UT receptors. Eur J Pharmacol 2004;492:113–6.
[4] Behm DJ, Stankus G, Doe CP, Willette RN, Sarau HM, Foley JJ, et al. The peptidic urotensin-II receptor ligand GSK248451 possesses less intrinsic activity than the low-efficacy partial agonists SB-710411 and urantide in native mammalian tissues and recombinant cell systems. Br J Pharmacol 2006;148:173–90.
[5] Camarda V, Guerrini R, Kostenis E, Rizzi A, Calo G, Hattenberger A, et al. A new ligand for the urotensin II receptor. Br J Pharmacol 2002;137:311–4.
[6] Camarda V, Song W, Marzola E, Spagnol M, Guerrini R, Salvadori S, et al. Urantide mimics urotensin-II induced calcium release in cells expressing recombinant UT receptors. Eur J Pharmacol 2004;498:83–6.
[7] Camarda V, Spagnol M, Song W, Vergura R, Roth AL, Thompson JP, et al. In vitro and in vivo pharmacological characterization of the novel UT receptor ligand [Pen^5,D-Trp^7,Dab^8]urotensin II$_{(4\text{-}11)}$ (UFP-803). Br J Pharmacol 2006;147:92–100.
[8] Castel H, Diallo M, Chatenet D, Leprince J, Desrues L, Schouft MT, et al. Biochemical and functional

characterization of high-affinity urotensin II receptors in rat cortical astrocytes. J Neurochem 2006;99:582–95.
[9] Chatenet D, Dubessy C, Leprince J, Boularan C, Carlier L, Ségalas-Milazzo I, et al. Structure–activity relationships on structural conformation of a novel urotensin II-related peptide. Peptides 2004;25:1819–30.
[10] Clozel M, Binkert C, Birker-Robaczewska M, Boukhadra C, Ding SS, Fischli W, et al. Pharmacology of the urotensin-II receptor antagonist palosuran (ACT-058362; 1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulfate salt): first demonstration of a pathophysiological role of the urotensin system. J Pharmacol Exp Ther 2004;311:204–12.
[11] Conlon JM, O'Harte F, Smith DD, Tonon MC, Vaudry H. Isolation and primary structure of urotensin II from the brain of a tetrapod, the frog *Rana ridibunda*. Biochem Biophys Res Commun 1992;188:578–83.
[12] Conlon JM, Tostivint H, Vaudry H. Somatostatin- and urotensin II-related peptides: molecular diversity and evolutionary perspectives. Regul Pept 1997;69:95–103.
[13] Coulouarn Y, Jégou S, Tostivint H, Vaudry H, Lihrmann I. Cloning, sequence analysis and tissue distribution of the mouse and rat urotensin II precursors. FEBS Lett 1999;457:28–32.
[14] Coulouarn Y, Lihrmann I, Jégou S, Anouar Y, Tostivint H, Beauvillain JC, et al. Cloning of the cDNA encoding the urotensin II precursor in frog and human reveals intense expression of the urotensin II gene in motoneurons of the spinal cord. Proc Natl Acad Sci USA 1998;95:15803–8.
[15] Delumeau JC, Marin P, Tence M, Cordier J, Glowinski J, Premont J. Synergistic regulation of cytosolic Ca^{2+} concentration by somatostatin and alpha-1-adrenergic agonists in mouse astrocytes. J Physiol Paris 1992;86:31–8.
[16] Elshourbagy NA, Douglas SA, Shabon U, Harrison S, Duddy G, Sechler JL, et al. Molecular and pharmacological characterization of genes encoding urotensin-II peptides and their cognate G-protein-coupled receptors from the mouse and monkey. Br J Pharmacol 2002;136:9–22.
[17] Feindt J, Becker I, Blomer U, Hugo HH, Mehdorn HM, Krisch B, et al. Expression of somatostatin receptor subtypes in cultured astrocytes and gliomas. J Neurochem 1995;65:1997–2005.
[18] Flohr S, Kurz M, Kostenis E, Brkovich A, Fournier A, Klabunde T. Identification of nonpeptidic urotensin II receptor antagonists by virtual screening based on a pharmacophore model derived from structure–activity relationships and nuclear magnetic resonance studies on urotensin II. J Med Chem 2002;45:1799–805.
[19] Gandolfo P, Patte C, Leprince J, Thoumas JL, Vaudry H, Tonon MC. The stimulatory effect of the octadecaneuropeptide (ODN) on cytosolic Ca^{2+} in rat astrocytes is not mediated through classical benzodiazepine receptors. Eur J Pharmacol 1997;322:275–81.
[20] Kinney WA, Almond Jr HR, Qi J, Smith CE, Santulli RJ, de Garavilla L, et al. Structure–function analysis of urotensin II and its use in the construction of a ligand-receptor working model. Angew Chem Int Ed Engl 2002;41:2940–4.
[21] Labarrère P, Chatenet D, Leprince J, Marionneau C, Loirand G, Tonon MC, et al. Structure–activity relationships of human urotensin II and related analogues on rat aortic ring contraction. J Enzyme Inhib Med Chem 2003;18:77–88.
[22] Leprince J, Chatenet D, Dubessy C, Fournier A, Pfeiffer B, Scalbert E, et al. Structure–activity relationships of urotensin II and urotensin II-related peptide. Peptides 2008;29:658–73.
[23] Liu Q, Pong SS, Zeng Z, Zhang Q, Howard AD, Williams Jr DL, et al. Identification of urotensin II as the endogenous ligand for the orphan G-protein-coupled receptor GPR14. Biochem Biophys Res Commun 1999;266:174–8.
[24] Malagon MM, Molina M, Gahete MD, Duran-Prado M, Martinez-Fuentes AJ, Tonon MC, et al. Urotensin II and urotensin II-related peptide activate somatostatin receptor subtypes 2 and 5. Peptides; Submitted for publication.
[25] Marchese A, Heiber M, Nguyen T, Heng HH, Saldivia VR, Cheng R, et al. Cloning and chromosomal mapping of three novel genes, GPR9, GPR10, and GPR14, encoding receptors related to interleukin 8, neuropeptide Y, and somatostatin receptors. Genomics 1995;29:335–44.
[26] Masmoudi O, Gandolfo P, Tokay T, Leprince J, Ravni A, Vaudry H, et al. Somatostatin down-regulates the expression and release of endozepines from cultured rat astrocytes via distinct receptor subtypes. J Neurochem 2005;94:561–71.
[27] Mori M, Sugo T, Abe M, Shimomura Y, Kurihara M, Kitada C, et al. Urotensin II is the endogenous ligand of a G-protein-coupled orphan receptor, SENR (GPR14). Biochem Biophys Res Commun 1999;265:123–9.
[28] Nehring RB, Meyerhof W, Richter D. Aspartic acid residue 124 in the third transmembrane domain of the somatostatin receptor subtype 3 is essential for somatostatin-14 binding. DNA Cell Biol 1995;14:939–44.
[29] Nothacker HP, Wang Z, McNeill AM, Saito Y, Merten S, O'Dowd B, et al. Identification of the natural ligand of an orphan G-protein-coupled receptor involved in the regulation of vasoconstriction. Nat Cell Biol 1999;1:383–5.
[30] Pearson D, Shively JE, Clark BR, Geschwind II, Barkley M, Nishioka RS, et al. Urotensin II: a somatostatin-like peptide in the caudal neurosecretory system of fishes. Proc Natl Acad Sci USA 1980;77:5021–4.
[31] Rossowski WJ, Cheng BL, Taylor JE, Datta R, Coy DH. Human urotensin II-induced aorta ring contractions are mediated by protein kinase C, tyrosine kinases and Rho-kinase: inhibition by somatostatin receptor antagonists. Eur J Pharmacol 2002;438:159–70.
[32] Saetrum Opgaard O, Nothacker H, Ehlert FJ, Krause DN. Human urotensin II mediates vasoconstriction via an increase in inositol phosphates. Eur J Pharmacol 2000;406:265–71.
[33] Song W, McDonald J, Camarda V, Calo G, Guerrini R, Marzola E, et al. Cell and tissue responses of a range of urotensin II analogs at cloned and native urotensin II receptors; evidence for coupling promiscuity. Naunyn Schmiedebergs Arch Pharmacol 2006;373:148–57.
[34] Strnad J, Hadcock JR. Identification of a critical aspartate residue in transmembrane domain three necessary for the binding of somatostatin to the somatostatin receptor SSTR2. Biochem Biophys Res Commun 1995;216:913–21.
[35] Sugo T, Murakami Y, Shimomura Y, Harada M, Abe M, Ishibashi Y, et al. Identification of urotensin II-related peptide as the urotensin II-immunoreactive molecule in the rat brain. Biochem Biophys Res Commun 2003;310:860–8.
[36] Tostivint H, Joly L, Lihrmann I, Parmentier C, Lebon A, Morisson M, et al. Comparative genomics provides evidence for close evolutionary relationships between the urotensin II and somatostatin gene families. Proc Natl Acad Sci USA 2006;103:2237–42.
[37] Ziltener P, Mueller C, Haenig B, Scherz MW, Nayler O. Urotensin II mediates ERK1/2 phosphorylation and proliferation in GPR14-transfected cell lines. J Recept Signal Transduct Res 2002;22:155–68.